Experimental aerosol transmission of *Actinobacillus pleuropneumoniae* to pigs

Jean-Luc Jobert, Chantal Savoye, Roland Cariolet, Marylène Kobisch, and François Madec

Abstract

In order to demonstrate the possible role of aerosol in the transmission of *Actinobacillus pleuropneumoniae*, an experiment including 18 specific pathogen-free (SPF), 10-week-old piglets, randomly distributed into 2 adjacent units, was carried out. In these facilities, air was forced through absolute filters to prevent any contact with infectious agents. During the first 6 d post inoculation, the 2 units were connected by a rectangular opening and the air circulation was forced by the ventilation system from unit A (inoculated pigs) to unit B (non-inoculated pigs). The *A. pleuropneumoniae* strain (biovar 1 serovar 9) was isolated in France from an outbreak of porcine pleuropneumonia. Two different infecting doses, 10^7 cfu/animal and 10^8 cfu/animal, were inoculated by intranasal route in 6 pigs of unit A. The infection spread quickly from the inoculated pigs to the non-inoculated pigs. Clinical signs were acute during the 4 d post inoculation: hyperthermia, respiratory distress and, sometimes, death (6 pigs of the unit A and 2 pigs of the unit B). All pigs seroconverted against *A. pleuropneumoniae* serovar 9 within 2 weeks. Lung lesions were severe: fibrinous pleurisy and lung hemorrhages in the acute stage, pleural adherences and focal pulmonary necrosis in the chronic stage. *Actinobacillus pleuropneumoniae* was isolated from the tonsils and/or lungs in 16 animals. It could be also isolated from the air of the experimental unit. This study showed that *A. pleuropneumoniae* was readily transmitted through aerosol over a distance of at least 2.5 m.

Résumé

*Une expérience fut réalisée à l'aide de 18 porcelets exempts de pathogènes spécifiques âgés de 10 semaines, répartis au hasard dans deux pièces adjacentes, afin de démontrer le rôle possible des aérosols dans la transmission d'*Actinobacillus pleuropneumoniae. *L'alimentation en air des bâtiments utilisés était filtrée afin de prévenir tout contact avec des agents infectieux. Au cours des six premiers jours suivant l'inoculation, une ouverture entre les deux pièces a permis à l'air de circuler de la pièce A (porcs inoculés) vers la pièce B (porcs non-inoculés). La souche d'*A. pleuropneumoniae *utilisée (biovar 1 sérovar 9) fut isolé en France lors d'un épisode de pleuropneumonie porcine. Deux doses différentes, 10^7 ufc/animal et 10^8 ufc/animal, furent inoculées par voie intranasale à 6 porcs de l'unité A. L'infection s'est répandue rapidement des porcs inoculés aux porcs non-inoculés. Des signes cliniques aigus furent observés au cours des 4 jours suivant l'inoculation : hyperthermie, détresse respiratoire, et parfois la mort (6 porcs de la pièce A et 2 porcs de la pièce B). Une séroconversion envers* A. pleuropneumoniae *sérovar 9 fut observée en 2 semaines ou moins chez tous les animaux. Les lésions pulmonaires étaient sévères avec une pleurésie fibrineuse et des hémorragies pulmonaires dans la période d'infection aiguë, et des adhérences pleurales et de la nécrose pulmonaire focale dans la période d'infection chronique.* Actinobacillus pleuropeumoniae *fut isolé des amygdales et/ou des poumons de 16 animaux. La bactérie fut également isolée à partir de l'air des pièces d'expérimentation. Cette étude a démontré qu'*A. pleuropneumoniae *fut transmis facilement par aérosol sur une distance d'au moins 2,5 m.*

(Traduit par docteur Serge Messier)

Introduction

Actinobacillus pleuropneumoniae is a very common microorganism in industrial pig herds all over the world. The disease generally affects growing/fattening pigs (2 to 6 mo old), though all life stages are sensitive (1,2). The economical impact is of great importance, mainly because of mortality, cost of treatment, but also growth retardation (3–5). According to environmental conditions (overcrowding, transportation, mixing), infecting dose and strain virulence, clinical development can be peracute, acute, subacute, or chronic (6,7). The main vehicle of the pathogen remains the pig itself (6,8). Some authors have demonstrated a direct contamination by nose-to-nose contact or via fine droplets over short distances (6,9). In the past, few studies have been focused on airborne transmission of swine pathogens. On the basis of epidemiological data, swine influenza virus (10), Aujeszky's disease virus (11), and *Mycoplasma hyopneumoniae* (12) seem to diffuse in the air. The purpose of this study was to evaluate the experimental airborne transmission of *A. pleuropneumoniae* over a distance of a few metres.

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Materials and methods

Experimental design

Two adjacent experimental units were used in this trial. Very strict biosecurity measures were implemented in order to avoid undesirable contamination of the pigs: existence of an air filtration system and airlocks for each unit, unit-specific clothes, and compulsory showering before and after visiting the pigs. Each unit was 67 m^3 in size and had a separate and adjustable fan ventilation system. The airflow direction and rate could be chosen according to the development of the trial. A first unit (unit A) included 2 pens (numbered 1 and 2) of 5 pigs each. A second unit (unit B) included 2 pens (numbered 3 and 4) of 4 pigs each. The pens were wire flatdecks raised on legs (0.40 m height). The concrete surface below was cleaned every day. Before the experimental infection, a rectangular window (0.40 m × 0.68 m) was opened between the units and an oriented airflow was set up through a suitable adjustment of the ventilation system: air input into unit A exclusively and air extraction from unit B (Figure 1). The airflow (about 65 m^3/h/pig) was maintained for 6 d post inoculation. On Day 6, the window between the units was closed and each of them recovered its own ventilation system (Figure 2). The distance between the animals of units A and B ranged from 2.50 to 5 m.

Animals

Pigs were specific pathogen-free (SPF) and were born in our own experimental SPF herd (13). They were experimentally infected at 10 wk old, about 37 kg body weight (BW). In unit A, 3 pigs per pen were randomly selected for the experimental inoculation. The 4 last pigs of unit A served as contact pigs. The 8 pigs of unit B were sentinels.

A. pleuropneumoniae strain and experimental infection

Pigs were intranasally (IN) infected with *A. pleuropneumoniae* strain 4915, cultured 6 h on nicotinamide adenine dinucleotide (NAD; 10 µg/mL)-supplemented PPLO medium. This strain, biovar 1 serovar 9, was isolated from a field case of pleuropneumonia in France. Two different doses were employed in unit A in order to observe a potential gradation in lesions and clinical signs in inoculated pigs. Three pigs in pen 1 were infected with 0.5 mL/nostril of a 10^7 cfu/mL suspension. In pen 2, 3 pigs were infected in the same way, with a 10^8 cfu/mL suspension.

Clinical monitoring

Daily clinical examinations consisted in taking rectal temperature and looking for symptoms such as anorexia, asthenia, tachypnea, dyspnea, cough, cyanosis, nasal discharge or foaming. During the 4 d following the experimental infection, the inoculated and contact pigs were observed twice daily.

Samples

Blood samples were taken for serological analysis on Day −5, then on Days 6, 13, 20, and 22 (pigs of unit A) or 27 (pigs of unit B). Sera were stored at −20°C ± 2°C, while waiting for analysis. Serological tests were realised by an ELISA technique using long chain purified

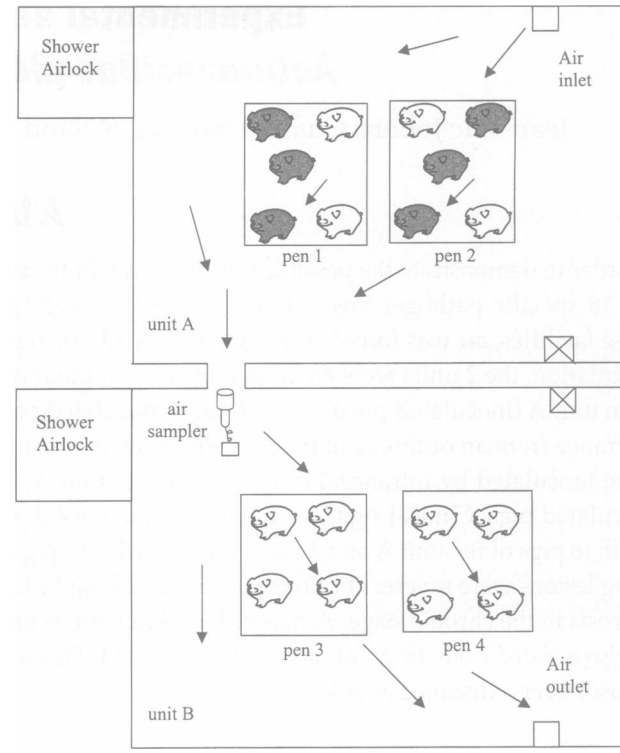

Figure 1. Experimental design of the pig accommodations during the period Day −1–Day 6. Arrows indicate the airflow direction. In each pen, the number of pigs illustrated corresponds with the situation on the day of inoculation. The inoculated pigs are shaded. Size of the *A. pleuropneumoniae* inoculum: pen 1: 10^7 cfu/pig; pen 2: 10^8 cfu/pig.

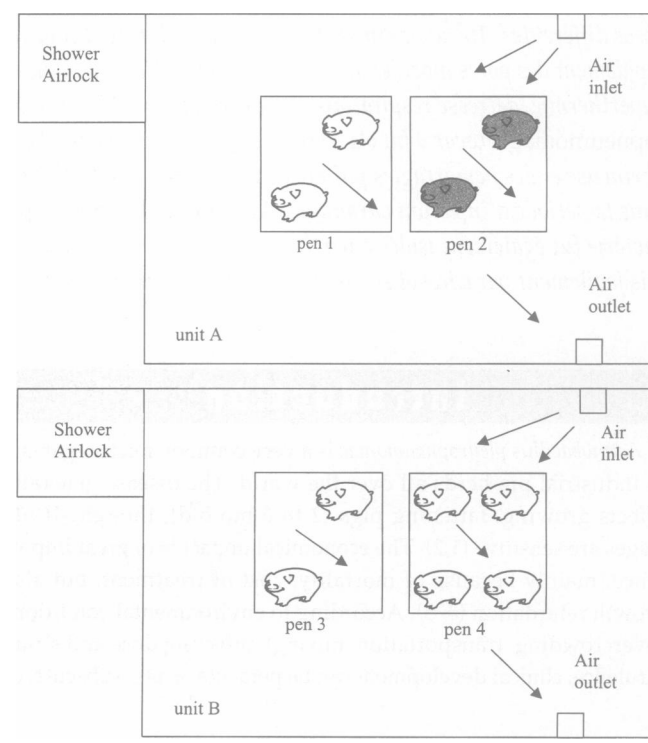

Figure 2. Experimental design after Day 6. Arrows indicate the airflow direction. In the figure, the number of pigs in each pen corresponds to the situation from Day 6 to the end of the trial. The inoculated pigs are shaded.

Table I. Clinical, pathological, bacteriological and serological results of infected, contact and sentinel pigs

# Pig	Group	Fever period (> 40°C)	Death (d after infection)	Lung lesions	App isolation		Time to Seroconversion (d)
					lungs	tonsils	
5956	Infected with 10^7 cfu (unit A-pen 1)	Day 1	Day 1	Fibrinous and hemorraghic pleuropneumonia	yes	no	ND[a]
5992	Infected with 10^7 cfu (unit A-pen 1)	Day 1	Day 1	Fibrinous and hemorraghic pleuropneumonia	yes	yes	ND
5995	Infected with 10^7 cfu (unit A-pen 1)	Day 1	Day 1	Fibrinous and hemorraghic pleuropneumonia	yes	yes	ND
5969	Infected with 10^8 cfu (unit A-pen 2)	Day 1	Day 1	Fibrinous and hemorraghic pleuropneumonia	yes	yes	ND
5982	Infected with 10^8 cfu (unit A-pen 2)	Day 1–7	Day 22	Fibrous pleurisy	yes	yes	6 d
5983	Infected with 10^8 cfu (unit A- pen 2)	Day 1–9	Day 22	Fibrous pleurisy	yes	yes	6 d
5963	Contact (Unit A-pen 1)	Day 2–7	Day 22	Fibrous pleurisy, pulmonary necrosis	yes	yes	13 d
5988	Contact (unit A-pen 1)	Day 1	Day 22	Fibrous pleurisy	no	no	13 d
5958	Contact (unit A-pen 2)	Day 1–2	Day 3	Fibrinous and hemorraghic pleuropneumonia	yes	yes	ND
5978	Contact (unit A-pen 2)	Day 1–3	Day 4	Fibrinous and hemorraghic pleuropneumonia	yes	yes	ND
5941	Sentinel (unit B-pen 3)	Day 1 & 4	Day 27	Fibrous pleurisy, pulmonary necrosis	yes	no	6 d
5951	Sentinel (unit B-pen 3)	Day 1–5	Day 27	absence	yes	yes	13 d
5986	Sentinel (unit B-pen 3)	Day 1–2	Day 3	Fibrinous and hemorraghic pleuropneumonia	yes	no	ND
5991	Sentinel (unit B-pen 3)	Day 1	Day 2	Fibrinous and hemorraghic pleuropneumonia	yes	yes	ND
5942	Sentinel (unit B-pen 4)	Day 1–7	Day 27	Fibrous pleurisy, pulmonary necrosis	no	no	13 d
5953	Sentinel (unit B-pen 4)	Day 1–6, Day 9–10	Day 27	Fibrous pleurisy	yes	yes	6 d
5964	Sentinel (unit B-pen 4)	absence	Day 27	absence	no	yes	13 d
5973	Sentinel (unit B-pen 4)	Day 1–3, Day 5–6	Day 27	Fibrous pleurisy	yes	yes	6 d

ND — Not determined

App — *Actinobacillus pleuropneumoniae*

lipopolysaccharides (LC-LPS), specific for serogroup 1-9-11 (14). The positive threshold was fixed at an optical density (OD) of 0.4. If mortality occurred, or after euthanasia, the pigs were necropsied and the thoracic organs were thoroughly examined. The pigs of units A and B were sacrificed on Days 22 and 27, respectively. A bacteriological exam was done on the tonsils and lung tissue, even in the cases where no gross lesions were observed. A selective based Columbia medium, enriched with blood (5%), NAD (0.5 mg/mL), yeast extract (5%), and supplemented with 1.13 µg/mL lincomycin and 64 µg/mL bacitracin was employed for *A. pleuropneumoniae* isolation. Colonies were biochemically confirmed as *A. pleuropneumoniae* (api 20E, Biomérieux) and serotyped by coagglutination test with a type-specific hyperimmune serum. From Day −1 to Day 6, 18 regular attempts to detect *A. pleuropneumoniae* in the air were performed with an air sampler (SAS model, Fisher Bioblock Scientific, Illkirch, France). This one includes only one sample stage using a Rodac plate (33 cm^2). Air is accelerated through small orifices at a rate of 180 L/min. The device was left in unit B at the entrance of airflow and pumped 900 L of air over each 5-minute use. The detection threshold was 1 viable microorganism/900 L. Since we were aiming for a specific qualitative measure of aerobiocontamination, a selective medium was used in the contact plates (same composition as above).

Results

The main clinical, pathological, bacteriological and serological findings are reported in Table I.

Symptoms

The difference in titration of the inocula seems to have a low influence on the magnitude of the clinical signs. Normal rectal temperature of a SPF growing pig is about 39.5°C. The 6 experimentally

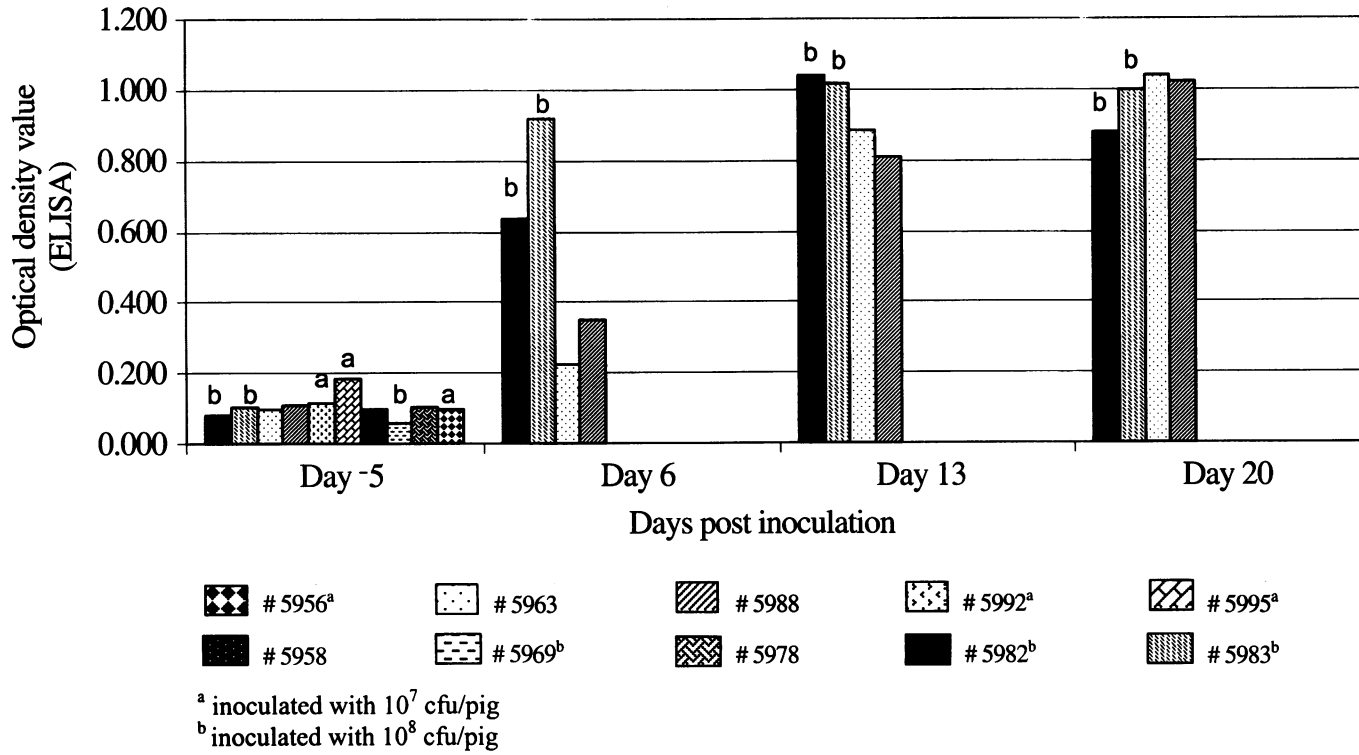

Figure 3. Antibody response in experimentally infected and contact pigs.

infected pigs displayed hyperthermia and prostration within 10 h of inoculation (41.1°C ± 0.4°C). In 15 to 22 h, 4 pigs died with respiratory distress (tachypnea, dyspnea and cyanosis). Among the survivors in pen 2, 1 pig (#5983), heavily breathless and cyanotic, received on Day 1 an ampicillin-based therapeutic (8.66 g/100 mL) to avoid death: 2 IM injections, each separated by 9 h, at a dose of 5 mg/kg (BW). In pen 1, the contact pigs exceeded 41°C in rectal temperature 16 to 40 h post inoculation, then displayed respiratory symptoms, and eventually recovered within a few days. In pen 2, the contact pigs showed hyperthermia 10 to 15 h post inoculation (> 40.8°C) and died on Days 3 and 4 with marked respiratory symptoms. Over the whole trial, only one sentinel did not show any clinical signs (pen 4: #5964). With the exception of this one, all other sentinels ran a high body temperature, within about 22 h of inoculation (40.9°C ± 0.8°C). These animals also presented severe weakness and respiratory symptoms (dyspnea, tachypnea) until Day 3. Two sentinels in pen 3 died 24 to 60 h post inoculation.

Post mortem examinations

Eight pigs died within 4 d of inoculation and had fibrinous and hemorrhagic pleuropneumonia lesions. Fibrin deposit and pulmonary hemorrhages were very severe, especially in starting acute phase. On Day 22 or 27 after inoculation, 2 pigs did not show any lung lesions (sentinels #5951 and #5964) and 8 others presented dry pleurisy. Focal necrosis in lung tissue was observed on 3 occasions in these latter pigs.

Bacterial culture

Tonsils and lungs — *Actinobacillus pleuropneumoniae* serovar 9 was isolated from tonsils and/or lungs in 16 pigs (10 in unit A, 6 in unit B). In 8 pigs with acute and fatal form of the disease, *A. pleuropneumoniae* serovar 9 was isolated from all lungs and from tonsils in 6 cases. On Day 22 or 27, *A. pleuropneumoniae* serovar 9 was isolated from both lungs and tonsils in 6 pigs, from lungs only in 1 pig, and from tonsils only in 1 pig. In 2 cases, *A. pleuropneumoniae* was not isolated from lungs or from tonsils. One sentinel pig showed definite clinical disorders for 5 d without macroscopic lung lesion; however, this pig bore *A. pleuropneumoniae* in lung tissue (at the diaphragmatic lobe) and tonsils. In the sentinel pig without any clinical signs, *A. pleuropneumoniae* serovar 9 was isolated from the tonsils.

Contact plates — Of 18 contact plates collected, 1 was positive for *A. pleuropneumoniae*. Indeed, only 2 colonies of *A. pleuropneumoniae* serovar 9 were recovered, at Day 3.

Antibody response

All the survivors synthesised antibodies against *A. pleuropneumoniae* serovar 9 within 13 d. Experimentally infected pigs became seropositive in less than 6 d. The 2 contact pigs seroconverted more slowly, within a period of 6–13 d (Figure 3). In the sentinel pigs, 3 were already positive by Day 6, while the 3 others became seropositive between 6 and 13 d after inoculation (Figure 4). The speed of seroconversion did not vary with the location of the animal in the unit.

Discussion

An IN inoculation of SPF pigs, at 10^7 or 10^8 cfu/pig, induced the same pathological pattern as observed in field outbreaks: rapid and high fever, exhaustion, respiratory collapse with tachypnea, dyspnea and cyanosis, just before death. These results confirm

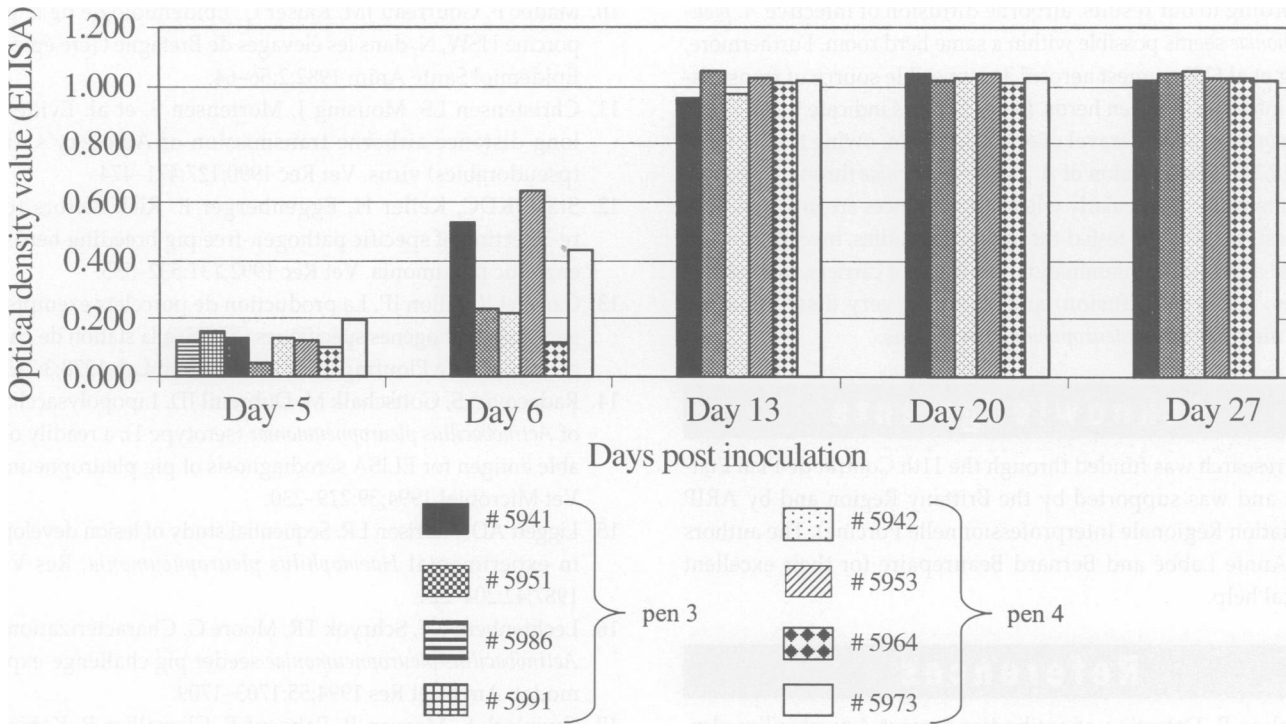

Figure 4. Antibody response in sentinel pigs.

previous works (2,6,7). Only 2 experimentally infected pigs developed a chronic form after a few days; in one of these, the condition improved due to antibiotic treatment. In the trial, the role of the 4 contact pigs was first to boost the infection burden, in case of very rapid death of the experimentally infected pigs. This objective was reached, as all of the pigs showed clinical signs and excreted *A. pleuropnemoniae*. Two contact pigs died on Days 3 and 4, and the 2 others recovered.

In fact, *A. pleuropneumoniae* spread very quickly and widely towards sentinel pigs. Clinical signs appeared in less than 24 h, and simultaneously affected a high proportion of sentinel pigs (7 of 8). This result is possible due to the rapid growth of the microorganism during acute outbreaks (15,16). The present study gives evidence of the airborne transmission of *A. pleuropneumoniae* and suggests that an acute outbreak yields a heavy bacterial burden in aerosol form. Thus in field situations, the air expired by sick animals (liquid part of aerosol) may serve as an important source of infection. Solid particles (skin scales; feed or feces particles) may play a role, but can not be assessed in this trial. *Actinobacillus pleuropneumoniae* was very rarely isolated from contact plates, though bacteria were effectively transported by the airflow. The concentration of the pathogen in the air, the volume of the air pumped by the air sampler, and the impinged surface might be too low to detect a large number of colonies. The high incidence of infection may account for the speed and generalization of the antibody response in sentinel pigs: all of them seroconverted within 6 to 13 d. A seroepidemiological study run by Guzylack et al (17) in *A. pleuropneumoniae* serovar 9-contaminated herds showed a more progressive seroconversion (6–8 wk). This divergence can be explained by a different immunogenic power of strains, lower contamination levels by *A. pleuropneumoniae*, and other, miscellaneous bacterial attacks in field conditions. Despite the important infecting burden of the aerosol, the amount of bacteria inhaled by each sentinel pig was variable. Pigs placed in pen 3, near the contaminated air arrival, may have received a higher infecting dose than pigs in pen 4, which may account for the early deaths in pen 3. In contrast, no pigs died in pen 4 and 1 pig proved to be non-susceptible. This latter pig never had hyperthermia but did develop an antibody response; this observation complies with previous reports (2,18). The pathogen was isolated from its tonsils 27 d after inoculation. This pig illustrates the asymptomatic carriage, reported elsewhere in field conditions (6,19). These healthy carriers are known to introduce and shed the bacteria in non-infected stocks (20,21). A stress may trigger an outbreak, initiated by the transmission of the pathogen from an immune carrier to susceptible animals (18).

The pigs of units A and B were 2.5 to 5 m apart, but the bacterial concentration in aerosol was enough to infect all the sentinel pigs at the same time. Recently, Toremorell et al (22) demonstrated the airborne diffusion of *A. pleuropneumoniae* over a short distance (1 m) under controlled experimental conditions. For this purpose, 2 chambers were made with aluminium and plexiglass (1.2 × 1.2 m) and connected together by a rectangular conduct (0.6 × 0.6 m). Ventilation was also forced from infected pigs to non-infected pigs (ventilation rate ranging from 4.8 to 12 $m^3/h/pig$). In the present study, the airborne contamination was observed over larger distances (> 2.50 m) and in a setup closer to field conditions with respect to room size and ventilation rate. In fattening units, air replacement rate usually depends on ambient temperature and pig weight. Recommended values for ventilation rates are near 40 $m^3/h/pig$ for 40 kg BW and can reach 70 $m^3/h/pig$ by the end of the finishing period.

According to our results, airborne diffusion of infective *A. pleuropneumoniae* seems possible within a same herd room. Furthermore, Fussing et al (23) suggest aerosol as a possible source of transmission of infection between herds. Other authors indicate that *A. pleuropneumoniae* is able to travel over 400 to 500 m, owing to dominant winds (8,24). Transmission of *A. pleuropneumoniae* through the air is difficult to prove, particularly when long distances are involved. This hypothesis needs to be tested through field studies, investigating all potential sources of contamination (purchase of carriers, inert or live vehicles, airborne diffusion) and applying very discriminatory typing methods of *A. pleuropneumoniae* strains.

Acknowledgments

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